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COMPONENTS OF JP-4..(U) NORTH DAKOTA STATE UNIV FARGO
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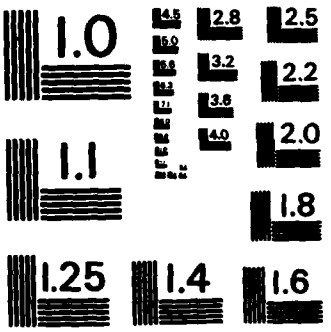

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September 30, 1978 - February 27, 1984

Title: Identification and Quantification of the Water-Solube Components of JP-4 and a Determination of their Biological Effects Upon Selected Freshwater Organisms

SUBMITTED BY:

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This final technical report includes a brief summary of research performed, results obtained, graduate students supported and theses written.

One paper published was of a technical nature and describes the use of reversephase C-18 minicolumns for concentrating water soluble hydrocarbons derived from JP-4 jet fuel. Another technical paper using the same technique as the first was used to concentrate water soluble hydrocarbons produced by

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running an outboard motor in water. Analytical methods used for hydrocarbon separation and identification was GC, GC/MS and HPLC.

The toxicity of toluene on the fathead minnow was the basis of three papers and a Ph.D. thesis. It was found that the embryo was as sensitive to toluene than was the protolarvae or adult fish. This was determined using 96-hr LC50 tests. A detailed embryonic study of the fathead embryo was performed. It was found that toluene produced the following terata: distorted embryonic axis, abnormal heart and circulatory system development, hydration and swelling of the pericardial coelom, hemorrhaging, an overall stunted appearance, microphthalmia, and a unique migration of the ventrally located yolk syncytial layer and its associated nuclei. An MS thesis was written on the effects of toluene on gill structure in the fathead minnow adult. Little effect of toluene on gill structure was noted.

A comparative study on the effects of administration of benzene, toluene and xylene isomeres on their in vitro metabolism and various drug metabolizing enzymes in rat liver, and the covalent binding of toluene to rat liver microsomes has resulted in one Ph.D. thesis and the preparation of three manuscripts for publication. Originator supplied terms includes

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Work Scope of Final Technical Report

(September 30, 1978 to February 27, 1984)

This report is divided into the following sections:

I. Abstracts of papers published during the tenure of this grant.

II. Abstracts of papers in press or submitted for publication.

III. Abstracts of papers in preparation for publication.

IV. List of graduate students supported and theses written.

V. List of papers and presented.

I. ABSTRACTS FROM PAPERS PUBLISHED AND SENT TO USAFOSR

A. Puyear, P. L., K. J. Fleckenstein, W. E. Montz, Jr., and J. D. Brammer. 1981. Use of reverse phase C-18 minicolumns for concentrating water-soluble hydrocarbons. Bull. Environm. Contam. Toxicol. 227, 790-797.

Reverse phase C-18 minicolumns (Sep-Pak) proved to be a useful tool for concentrating aliphatic hydrocarbons and alkylbenzenes from water. They were 5-10% less effecient than XAD-2 for concentrating some of the same aromatic hydrocarbons. This, however, is not a great reduction in efficiency when the ease and convenience of using these cartridges is considered. The C-18 paking material is rather hydrophobic. A useful extension of this technique for the concentrating of more water soluble hydrocarbons and organics would be to use minicolumns packed with more polar-bonded phases such as C-4 or C-2.

B. Devlin, E. W., J. D. Brammer and R. L. Puyear. 1982. Acute toxicity of toluene to three age groups of fathead minnows. Bull. Environ. Contam. Toxicol. 29:12-17.

The 96-hr LC50 for toluene in three age groups of the fathead minnow was determined. The toluene concentration that caused a 96-hr LC50 for embryos ranged from 55-72 mg/L, one day old post-hatch protolarvae 25-36 mg/L, and 30 day old fathead minnows 26-31 mg/L. The 96-hr LC50 was significantly higher for the embryos. The 96-hr LC50 values for the protolarvae and 30 day old minnows was the same.

C. Montz, W. E., Jr., R. L. Puyear and J. D. Brammer. 1982. Identification and quantification of water-soluble hyudrocarbons generated by two-cycle outboard motors. Arch. Environm. Contam. Toxicol. 11:561-565.

Equipment used in this reearch was purchased with funds provided by USAFOSR, NDSU, and USDA. Therefore, even though this research was not directly sponsored by USAFOSR it was cited as providing support.

A 7.0 horsepower (HP) and a 10.0 HP outboard motor were operated at 3,500 ± 200 rpm and 1,700 ± 200 rpm, respectively for 30 min in a 160 L tank of tapwater. Exhaust hydrocarbons were concentrated by passage through a C-18 reverse phase extraction column, and eluted with either ethylacetate or acetonitrile. Gas-liquid and/or high pressure liquid chromatographic procedures were used for identification and quantification of eight hydrocarbons. Identities were confirmed for seven of the compounds by GLC/mass spectrometry. Four additional hydrocarbons were tentatively identified with these procedures. Aromatic compounds composed the majority of the hydrocarbons detected; a few aliphatics were present in trace amounts.

D. Carter, F. D., R. L. Puyear and J. D. Brammer. 1984. Effects of aroclor 1254 treatment on the in vitro hepatic metabolism of toluene, aniline and aminopyrine in hybrid sunfish. Comp. Biochem. Physiol. Vol. 78C. No. 1, pp 137-140.

1. The in vitro metabolism of the volatile aromatic hydrocarbon toluene by enzymes associated with the 12,000 g supernatant fraction of hybrid sunfish (Lepomis macrocirus x L. cyanellus x L. gibbosus) liver homogenates was studied.

2. Aminopyrine demethylase (APDM) and aniline hydroxylase (AH) activities were measured.

3. Intramuscular injections of Aroclor 1254 (a polychlorinated biphenyl) produced significant increases in APDM and AH activities ($P < 0.1$, ANOVA). There were no significant changes in the metabolism of toluene, liver wet weights, or liver protein concentrations following treatment.

II. ABSTRACTS OF PAPERS IN PRESS OR SUBMITTED FOR PUBLICATION.

A. Devlin, E. W., J. D. Brammer and R. L. Puyear. 1985. Effect of toluene on fathead minnow Pimephales promelas Rafinesque development. Arch. Environm. Contam. Toxicol. (in press).

The effect of toluene on teleost development was investigated using the fathead minnow Pimephales promelas Rafinesque, a native North American Cyprinid. Live embryos as well as serial sections of paraffin-embedded embryos were examined to determine effects of the toxicant. Toluene concentrations utilized in this study (30-45 mg/l) were quite high and may be approached in the environment only under extreme conditions. Terata noted under these conditions included distorted embryonic axis, abnormal heart and circulatory system development, hydration and swelling of the pericardial coelom, hemorrhaging, an overall stunted appearance, microphthalmia, and a unique migration of the ventrally located yolk syncytial layer and its associated nuclei. Similarities between the effects of toluene and those of other environmental and chemical stressors are discussed.

B. Pathiratne, A., R. L. Puyear and J. D. Brammer. 1985. A comparative study of the effects on benzene, toluene and xylene on their in vitro metabolism and drug metabolizing enzymes in rat liver. J. Appl. Pharmacol. Toxicol. (submitted).

Male Sprague Dawley rats were injected ip with benzene, toluene or a mixture of xylene isomers at 20 mmol hydrocarbon/kg daily for 3 days. The effects of pre-exposure to these hydrocarbons had upon their own in vitro metabolism, as well as upon cytochrome P-450, NADPH-cytochrome c reductase, aminopyrine N-demethylase, aniline hydroxylase, glutathione, glutathione S-transferase, and UDP-glucuronyltransferase in liver were studied. Each hydrocarbon studied increased its own in vitro metabolism. Benzene had no effect on the metabolism of toluene or xylenes. Toluene and xylenes increased the metabolism of benzene, toluene, and xylenes. Cytochrome P-450 level was elevated by toluene and xylenes, but was not affected by benzene. NADPH-cytochrome c reductase was induced by all three hydrocarbons. Aminopyrine N-demethylase and aniline hydroxylase were induced by toluene and xylenes and were not affected by benzene. Glutathione level was elevated by benzene, decreased by xylenes, and not affected by toluene. Glutathione S-transferase was induced differentially by these hydrocarbons towards various substrates; towards 1-chloro-2,4-dinitrobenzene by benzene and toluene, towards 1,2-dichloro-4-nitrobenzene by benzene and xylenes and no effect towards 1,2-epoxy-3-(p-nitrophenoxy) propane by any hydrocarbons. UDP-glucuronyltrasferase was induced by benzene and toluene when o-aminophenol and phenol was used as the substrate. Xylenes had no effect. Benzene was more effective at inducing conjugation enzymes. Xylenes were more effective at inducing cytochrome

P-450 dependent enzymes. Toluene was equipotent at inducing both types of enzymes. The results indicate that the addition of methyl groups to the aromatic ring affects the enzymes induced by these monocyclic aromatic hydrocarbons.

III. ABSTRACT OF PAPERS IN PREPARATION FOR PUBLICATION OR BEING REVIEWED

A. Devlin, E.W., J. D. Brammer and R. L. Puyear. 1985. Prehatching development of the fathead development (Pimephales promelas Ref.). Being reviewed by Dr. Jim McKim at the Duluth EPA Laboratory

The fathead minnow, Pimephales promelas Raf. represents a classical model of teleostean embryogenesis. Its prehatching development has been divided into 32 stages, each representing an interval in the developmental continuum. Embryos were examined live and histologically under controlled laboratory conditions. Fertilization, early cleavage, epiboly, and organogenesis are very similar to that of other cyprinids except for the timing of the appearance of specific structures. Hatching was found to occur in approximately 120 hours post-fertilization at 25 C. Rapid embryonic development, coupled with a short generation time of 3-6 months under laboratory conditions, make it a useful native North American species for studies in experimental embryology.

B. Pathiratne, A., R. L. Puyear and J. D. Brammer. 1985. A headspace sampling method to study the in vitro metabolism of benzene, toluene, and xylene in rat liver. To be submitted to J. Appl. Pharmacol. Toxicol.

A headspace gas chromatographic method for the metabolic studies of benzene, toluene, and xylene using vial equilibration method of Sato and Nakajima (1978) is described. At the end of the incubation with hydrocarbon in a closed vial, the unreacted hydrocarbon in the head space was loaded into a 0.5 ml Teflon loop attached to a 6 port Valco valve using a vacuum. Then the hydrocarbon in the loop was injected onto the gas chromatographic column. Effect of incubation time, protein, and hydrocarbon concentration on their metabolism was studied. Toluene and xylene metabolism was induced by the pretreatment of rats with phenobarbital (75 mg/kg) or 3-methylcholanthrene (20 mg/kg) or Aroclor 1254 (75 mg/kg) for 3 days, whereas benzene metabolism was not induced by any of these pretreatments. Investigating metabolic rates of various subcellular fractions of liver reveals that glutathione and gluthathione S-transferase in cytosol are involved in metabolism of benzene and toluene by microsomes.

C. Pathiratne, A., R. L. Puyear, and J. D. Brammer. 1985. Activation of ^{14}C-toluene to covalent binding metabolites by rat liver microsomes. To be submitted to Drug Metabol. Disposition.

^{14}C-Toluene was incubated with rat liver microsomes in the presence of a NADPH generating system and metabolites were concentrated on cyclohexyl Bon Elute cartridges. The metabolites were separated by reverse phase high performance liquid chromatography and identified by comparing the retention time of known standards. Toluene was converted to ^{14}C-benzylalcohol (30.6%), ^{14}C-cresols (4.5%), and an unidentified ^{14}C-metabolite (0.6%). The radioactivity was found to preferentially

bind covalently to microsomal proteins and not to RNA. The binding was greatly diminished when microsomes were heat denatured. The binding was proportional to incubation time and microsomal protein concentration and required NADPH and molecular oxygen. Binding process was inhibited by carbon monoxide and SKF 525-A. When microsomes from phenobarbital and 3-methylcholanthrene treated rats were employed, the binding process was markedly enhanced to 8 and 4 folds respectively. The binding process was effectively diminished by the presence of reduced glutathione or cystein in the incubation mixture and was not affected by lysine. Styrene oxide greatly enhanced the binding process. UDP-glucuronic acid, superoxide dismutase and ascorbic acid also diminished the binding to some degree. It was concluded that toluene undergoes a hepatic microsomal monooxygenases mediated activation and the resultant reactive metabolites bind covalently to microsomal macromolecules, preferentially to proteins.

IV. LIST OF GRADUATE STUDENTS SUPPORTED AND THESES WRITTEN

Walter E. Montz, Jr. Outboard motor exhausts. I. Identification an quantification of the water-soluble hydrocarbons generated by the operation of two-cycle outboard motors. II. A baseline study of water-soluble hydrocarbons and boat use patterns on a recreational lake. M.S. 1980.

Edward W. Devlin. Developmental studies on the fathead minnow Pimephales promelas (Raf.). I. The prehatching development of the fathead minnow. II. The acute effects of toluene on three age groups of fathead minnows. III. The effect of toluene on the prehatching development of the fathead minnow. Ph.D. 1982.

Fred Drake Carter. Metabolism of toluene by liver 12,000 x g supernatants from rats and sunfish. M.S. 1983.

Russell Lawry. Normal morphology and the effect of toluene on the gill of the fathead minnow Pimephales promelas (Raf.). M.S. 1984.

Asoka Pathiratne. In vitro metabolism of benzene, toluene and xylene in rat liver. Ph.D. 1985.

V. LIST OF PAPERS PRESENTED

Montz, W. E., Jr., R. L. Puyear and J. D. Brammer. 1979. Identification and quantification of the water-soluble components of outboard motor exhaust and of gasoline in a North Dakota lake, and a determination of their biological effects upon seleted freshwater organisms. Presented at the North Dakota-South Dakota Joint Academy of Science Meeting, Aberdeen, SD.

Puyear, R. L., K. J. Fleckenstein and W. E. Montz, Jr. 1980. Use of Sep-Pak C 18 for concentrating water soluble hydrocarbons. Presented at the Minnesota Chromatography Forum, Minneapolis, MN.

Puyear, R. L., J. D. Brammer, E. W. Devlin and W. E. Montz, Jr. 1980. The identification of water soluble JP-4 hydrocarbons and bioassay of toluene using the fathead minnow, Pimephales promelas. Review of Air Force Sponsored Basic Research, San Antonio, Texas.

Devlin, E. W., J. D. Brammer, R. L. Puyear, and K. J. Fleckenstein. 1981. Effects of toluene on early development of the fathead minnows, Pimephales promelas. Air Force Sponsored Basic Research, Columbus, Ohio.

Puyear, R. L., A. Pathiratne, C. Medich and J. D. Brammer. 1983. Use of headspace sampling to study metabolism of benzene and toluene by various subcellular fractions of rat live. Presented at the Minnesota Chromatography Forum, Minneapolis, MN.

Devlin, E. W., J. D. Brammer and R. L. Puyear. 1984. Prehatching development of the fathead minnow. International Symposium on the Early Life History of Fishes. Univ. of British Columbia, Vancouver, BC. Canada.

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